Table 2 Atherosclerotic characteristics of the two study groups

	CAD (n=142)	Control (n=108)	p
Vascular structural change			
Extent of coronary artery disease, no. of vessel ≥50% in diameter, n (%)			<0.0001
0	0 (0)	108 (100)	
1	76 (54)	0 (0)	
2	25 (18)	0 (0)	
3	41 (29)	0 (0)	
Extent of carotid artery disease, IMTmax, mm	1.8±0.9	0.8±0.8	<0.0001
Vascular functional change			
Arterial stiffness,			
rt-baPWV, cm/sec	1873±531	1716±463	0.04
lt-baPWV, cm/sec	1870±520	1730±461	0.04

baPWV—brachial and ankle pulse wave velocity; CAD—coronary artery disease; IMT—intima-media thickness.

incidence of glucose intolerance and HDL-C levels in the two groups. Plasma levels of CRP were significantly higher, and plasma levels of adiponectin were significantly lower in the CAD group compared to the control group. The CAD group had significantly higher IMTmax and bilateral baPWV than the control group (Table 2).

The correlation between plasma adiponectin levels and other parameters

The linear relationships between plasma adiponectin levels, atherosclerotic vascular changes, and the other coronary risk factors are shown in Table 3. Plasma levels of adiponectin correlated negatively with glucose intolerance, fasting blood glucose, hemoglobin A_{1c}, TG, apolipoprotein (apo) B, apo C-II, BMI, CRP, and IMTmax, and correlated positively with HDL-C and apo A-I. Plasma adiponectin levels were also affected by sex, with higher values being found in the female patients. Plasma adiponectin concentrations were not correlated with PWV. Our finding that CAD was associated with a decrease in plasma adiponectin was reflected by a nonsignificant trend of lower adiponectin levels in proportion to the number of effected coronary arteries (1-vessel disease: 6.6±3.4 μg/mL; 2-vessel disease: 5.1±2.9 μg/mL; 3-vessel disease: 4.7±2.4 μg/mL).

The multiple linear regression analysis of the various biochemical and clinical parameters related to plasma adiponectin levels is shown in Table 4. This analysis revealed an independent significant

Table 3 Simple linear analysis of the relationship between plasma adiponectin levels and other clinical and biochemical factors

Independent variables	r	p
Age	0.10	0.11
Sex, men=0/women=1	0.15	0.02
Hypertension absent=0/present=1	−0.11	0.14
Systolic blood pressure	−0.01	0.85
Diastolic blood pressure	0.02	0.78
Glucose intolerance, absent=0/present=1	−0.35	<0.0001
Fasting blood glucose	−0.22	0.0004
Hemoglobin A_{1c}	−0.27	<0.0001
Total cholesterol	0.0008	0.90
Triglyceride	−0.27	<0.0001
LDL cholesterol	−0.11	0.07
HDL cholesterol	0.36	<0.0001
Apo A-I	0.20	0.002
Apo A-II	−0.05	0.42
Apo B	−0.19	0.003
Apo C-II	−0.13	0.04
Apo C-III	−0.11	0.07
Apo E	0.04	0.51
Smoking, absent=0/present=1	−0.02	0.70
Body mass index	−0.31	<0.0001
Creatinine	0.06	0.39
C-reactive protein	−0.38	<0.0001
Coronary artery disease, absent=0/present=1	−0.37	<0.0001
IMTmax	−0.37	<0.0001
Mean baPWV	−0.03	0.62

Apo—apolipoprotein; baPWV—brachial and ankle pulse wave velocity; HDL—high-density lipoprotein; IMT—intima-media thickness; LDL—low-density lipoprotein.

relationship between adiponectin and either glucose intolerance, CRP, BMI, HDL-C, IMTmax, or the presence of CAD.

We examined the relationship between atheromatous vascular changes and clinical parameters closely related to adiponectin. CRP, glucose

Table 4 Multiple linear regression analysis of independent determinants of plasma adiponectin levels

Independent variables	Regression coefficient	S.E. of regression coefficient	p
Intercept	15.36	2.28	<0.001
Glucose intolerance	−2.01	0.49	0.0001
C-reactive protein	−9.22	2.73	0.0009
Body mass index	−0.26	0.08	0.002
HDL cholesterol	0.04	0.02	0.02
IMTmax	−0.67	0.29	0.02
Coronary artery disease	−1.20	0.56	0.03
Triglyceride	−0.004	0.004	0.29
Female sex	0.39	0.52	0.46

HDL—high-density lipoprotein; IMT—intima-media thickness.

Table 5 Simple linear analysis between atheromatous vascular changes and clinical parameters closely associated with adiponectin

Independent variables	Adiponectin r (p)	IMTmax r (p)	CAD r (p)
C-reactive protein	−0.38 (<0.0001)	0.32 (<0.0001)	0.32 (<0.0001)
Glucose intolerance	−0.35 (<0.0001)	0.21 (0.0011)	0.26 (<0.0001)
HDL cholesterol	0.36 (<0.0001)	−0.23 (0.0002)	−0.25 (0.0001)
Body mass index	−0.31 (<0.0001)	0.03 (0.64)	0.06 (0.37)

CAD—coronary artery disease; HDL—high-density lipoprotein; IMT—intima-media thickness.

intolerance, and HDL-C that correlated with adiponectin were inversely correlated with IMTmax and CAD (Table 5). CRP was negatively correlated with HDL-C (r=−0.24, p=0.0002) and positively correlated with glucose intolerance (r=0.15, p=0.01).

Discussion

Our results confirm the findings of other studies that have shown that plasma adiponectin levels are lower in males and in patients with CAD, obesity, diabetes, or hypertriglyceridemia and are higher in subjects with normal or elevated levels of HDL-C [9,21]. Weyer et al. found that plasma adiponectin levels were also lower in patients with IGT as well as diabetes compared with normal subjects [22]. On the basis of these results, we classified individuals with either diabetes or IGT as being glucose intolerant. The significant correlations to adiponectin were not necessarily strong because the r-values were all less than 0.4 in Table 3. However, r-values observed in previous studies were not as high as our results [9,21,23].

It is well established that a low HDL-C level is a major independent risk factor for CAD [24,25]. Our finding that HDL-C was an independent determinant of plasma adiponectin level may have an important role in the development of atherogenesis. While the mechanism underlying the observed association between adiponectin and dyslipidemia is currently unknown, administration of adiponectin in obese mice was reported to decrease TG content in muscle and liver accompanied by an improvement in insulin sensitivity [15,26]. In our study, however, plasma adiponectin levels were more closely correlated with HDL-C than with TG, indicating that further studies are required to elucidate whether adiponectin influences atherogenesis by its effects on dyslipidemia and/or HDL-C metabolism.

Studies in experimental animals have shown that adiponectin has the potential to inhibit neointimal formation [14,15]. The observation that plasma adiponectin significantly suppressed the progression of atheromatous lesions in apo E-deficient mice provides additional evidence of such an action [27]. One explanation for the correlation we observed between plasma adiponectin levels, IMTmax, and the presence of CAD may be that adiponectin targets atherogenic plaques, resulting in consumption of the protein in the circulating plasma. An alternative possibility is that the atherogenic process may be accelerated in patients with low plasma levels of adiponectin. We have previously reported that adiponectin is inversely related to plasminogen activator inhibitor type 1 (PAI-1) in patients with stable exertional angina [28]. Whereas, we have also shown that PAI-1 contributes to the IMTmax of coronary arteries [29]. Therefore, adiponectin may be deeply involved in vascular structural changes through PAI-1.

Inflammatory structural changes to arteries such as endothelial injury and lipid-laden foam cells are known to be modulated by adiponectin [10,11]. The mechanical properties of elastic arteries may also be influenced by adiponectin inhibiting functional changes such as the proliferation and migration of vascular smooth muscle cells [12]. Therefore, there is increasing evidence that adiponectin may suppress both atheromatous and sclerotic vascular processes. The present study demonstrated that adiponectin was involved in the development of atheroma rather than sclerosis, and that adiponectin levels paralleled plasma CRP concentrations in humans. The CRP mRNA is expressed in human adipose tissue, and an inverse correlation is observed between the CRP and adiponectin mRNA levels in human adipose tissue [23]. We have demonstrated that CRP as well as glucose intolerance and HDL-C are common mediators between adiponectin and atheromatous vascular changes, which are contrary to each other. Furthermore, CRP was negatively correlated with HDL-C and positively correlated with glucose intolerance. Taken together, the exacerbation of atherogenesis may be involved in a decrease of adiponectin through abnormal glyco- and lipid-metabolism by promoting inflammation.

Interpretation of the findings of our study was limited by the fact that we were unable to ascertain whether IMT of the carotid artery and PWV reflected separate atheromatous or sclerotic vascular changes, respectively. This limitation arose as it is

well established that atheroma may be accompanied by sclerotic changes and that it is difficult to distinguish between these two disease processes. Notwithstanding these difficulties, we consider PWV to be used as an index of arterial stiffness based on the findings of previous studies in animal models and human subjects that support the accuracy of this method [30,31]. There is also general consensus that structural changes in the carotid artery can be detected by measuring IMT that provides an index of atheroma, while distensibility of the arteries can be assessed by functional changes of the PWV considered to be an index of sclerosis.

In conclusion, the determination of plasma adiponectin levels provides a new plasma marker of atheromatous vascular changes. Adiponectin may contribute to inhibition of atherogenesis by improving abnormal glyco- and lipid-metabolism involved in inflammation.

Acknowledgements

We gratefully acknowledge the technical assistance of Sachiyo Tanaka and Megumi Tsukamoto. This work was supported in part by grants for Clinical Vascular Function, Japan, Research Meeting on Hypertension and Atherosclerosis, Japan, and by grants-in-aid B(2)-14770319, C(1)-12203001, C(2)-13670727, and 14770318 from the Ministry of Education, Science, and Culture, Japan and the Smoking Research Foundation, Japan.

References

[1] Aschoff L. Introduction. In: Cowdry EV, editor. *Arteriosclerosis: a survey of the problem*. New York: MacMillan; 1933. p. 1–18.

[2] Blankenhorn DH, Kramsch DM. Reversal of atherosis and sclerosis: the two components of atherosclerosis. *Circulation* 1989;**79**:1–7.

[3] Davies MJ. A macro and micro view of coronary vascular insult in ischemic heart disease. *Circulation* 1990;**82(Suppl II)**:38–46.

[4] Feher MD. Lipid lowering to delay the progression of coronary artery disease. *Heart* 2003;**89**:451–8.

[5] Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 1983;**68**:50–8.

[6] Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application studies. *Hypertension* 1995;**26**:485–90.

[7] Taniwaki H, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, et al. Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. *Diabetes Care* 1999;**22**:1851–7.

[8] London GM, Marchais SJ, Safar ME, Genest AF, Guerin AP, Metivier F, et al. Aortic and large artery compliance in end-stage renal failure. *Kidney Int* 1990;**37**:137–42.

[9] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999;**257**:79–83.

[10] Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-κB signaling through a cAMP-dependent pathway. *Circulation* 2000;**102**:1296–301.

[11] Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 2001;**103**:1057–63.

[12] Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 2002; **105**:2893–8.

[13] Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999;**100**:2473–6.

[14] Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 2000;**32**:47–50.

[15] Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 2002;**277**:25863–6.

[16] Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertens Res* 2002;**25**:359–64.

[17] Suzuki E, Kashiwagi A, Nishio Y, Egawa K, Shimizu S, Maegawa H, et al. Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. *Diabetes Care* 2001;**24**:2107–14.

[18] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;**105**:1135–43.

[19] Report of a WHO Study Group. Prevention of diabetes mellitus: World Health Org., Geneva. 1994. World Health Organization Tech. Rep. Ser., No. 844.

[20] Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem* 1999;**45**:2136–41.

[21] Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;**20**:1595–9.

[22] Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001;**86**: 1930–5.

[23] Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 2003;**107**:671–4.

[24] Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults M. Executive summary of the third report of the national cholesterol education

program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adults treatment panel III). *JAMA* 2001;**285**:2486–97.

[25] Despres JP, Lemieux I, Dagenais GR, Cantin B, Lamarche B. HDL-cholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. *Atherosclerosis* 2000; **153**:263–72.

[26] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001;**7**:941–6.

[27] Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002;**106**:2767–70.

[28] Maruyoshi H, Kojima S, Funahashi T, Miyamoto S, Hokamaki H, Soejima H, et al. Adiponectin is inversely related to plasminogen activator inhibitor type 1 in patients with stable exertional angina. *Thromb Haemost* 2004;**91**:1026–30.

[29] Miyao Y, Fujimoto K, Kugiyama K, Kawano H, Hirai N, Sugiyama S, et al. Plasminogen activator inhibitor contributes to the coronary wall thickening in patients with angiographically normal coronary. *Thromb Res* 2003;**112**: 123–9.

[30] Farrar DJ, Bond MG, Riley WA, Sawyer JK. Anatomic correlates of aortic pulse wave velocity and carotid artery elasticity during atherosclerosis progression and regression in monkeys. *Circulation* 1991;**83**:1754–63.

[31] Wada T, Kodaira K, Fujishiro K, Maie K, Tsukiyama E, Fukumoto T, et al. Correlation of ultrasound-measured common carotid artery stiffness with pathological findings. *Arterioscler Thromb Vasc Biol* 1994;**14**:479–82.

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DIABETES RESEARCH AND CLINICAL PRACTICE
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Lifestyle modification improves risk factors in type 2 diabetes relatives

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Received 25 February 2004; received in revised form 17 May 2004; accepted 23 July 2004
Available online 8 October 2004

Abstract

Aims: To investigate the short-term (16 weeks) effect of lifestyle intervention on insulin sensitivity, anthropometric and metabolic variables in non-diabetic first-degree relatives of type 2 diabetic patients (FDR).
Methods: Seventy-seven (49 male, 28 female) FDR were allocated to one of three groups, diet (D-group: $n = 25$), diet and exercise (DE-group; $n = 30$) or control group (C-group: $n = 22$). Lifestyle counselling was based on current nutrition recommendations, including increased intake of fatty fish and low glycaemic index foods. Group counselling was given on two occasions with follow-up through telephone interviews every 10 days. Assessments included insulin sensitivity index (S_I), anthropometry, lipid parameters, circulating leptin and adiponectin levels.
Results: The D-group reduced total cholesterol (−0.31 mmol/l, $P = 0.024$), LDL cholesterol (−0.22 mmol/l, $P = 0.021$) and apolipoprotein B (−9.5 mg/dl, $P = 0.009$) levels, whereas the DE-group decreased body weight (−2.1%, $P = 0.030$) and waist circumference (−3.0 cm, $P < 0.001$) versus controls. A 13% reduction in fasting insulin was observed in the DE-group, but no significant improvement in S_I in D-group or DE-group was observed. A subgroup, adherent to diet and who increased exercise, significantly improved S_I and lipid profile.
Conclusions: The improved metabolic risk profile in FDR suggests that lifestyle changes can be effective in individuals at high risk to develop type 2 diabetes and cardiovascular disease.

Keywords: Diet; Exercise; Intervention; Relatives; Insulin resistance

1. Introduction

First-degree relatives of type 2 diabetic patients (FDR) have an increased risk of developing type 2

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doi:10.1016/j.diabres.2004.07.023

diabetes during their lifetime [1]. Therefore, they have been extensively studied and non-diabetic FDR show several metabolic abnormalities compared to subjects without a family history of diabetes. The most studied metabolic variables are insulin sensitivity and beta-cell function, which both have been reported to be reduced in this group [2–5]. Another affected variable is glucose effectiveness [6]. In addition to abnormal glucose metabolism, some studies have suggested disturbances in lipid metabolism such as increased triglycerides, reduced HDL and increased apolipoprotein B [7,8]. However, in these studies, the relatives had a higher BMI than the control group. Higher circulating leptin [9] and lower circulating adiponectin levels [10] have also been reported in FDR compared to controls.

Beneficial effects of changes in diet and exercise were shown recently in obese subjects with impaired glucose tolerance [11,12]. However, metabolic effects of lifestyle change have not been studied in FDR. They constitute a risk group for type 2 diabetes and coronary heart disease [8], and therefore a potential target group for preventive strategies. FDR have been reported to have a diet with larger amounts of total fat and saturated fat than persons without a family history of type 2 diabetes [13]. These dietary factors may promote obesity, insulin resistance [14] and other coronary heart disease risk factors [15].

The aim of this controlled lifestyle intervention trial was to evaluate the short-term effect on insulin sensitivity, metabolic and anthropometric variables in non-diabetic FDR. The two counselling strategies tested were diet alone and the combination of diet and increased physical activity, both with intensive follow-up. In addition, metabolic changes were also evaluated in those subjects who succeeded best with lifestyle intervention.

2. Materials and methods

2.1. Participants and study design

The goal was to include 90 FDR participants (allowing for a drop-out of 15 persons). Fig. 1 shows the flow diagram describing screening, inclusion and intervention in FDR. Baseline data were collected during two visits to our laboratory. We considered a participant eligible for inclusion if blood sample results were normal for liver function tests, electrolytes and hemoglobin as well as a medical history free from endocrine and cardiovascular diseases. An electrocardiogram was performed to assure there were no contraindications for increased exercise. Exclusion criteria were diabetes mellitus [16] as indicated by an oral glucose tolerance test (OGTT, 75 g glucose), body mass index (BMI) >35 kg/m^2 and diseases or medications affecting glucose or lipid metabolism. Seventy-seven non-diabetic volunteers (49 male and 28 female) aged 25–55 years were allocated to one of the three intervention groups by the method of minimization [17], matching for gender, age, BMI, impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) [16]. For practical reasons, enrolment (screening) and treatment allocation was performed at three time points: cohort 1 (33 subjects) in November 1997, cohort 2 (42 subjects) in April 1998 and cohort 3 (25 subjects) April 1999. The recruitment and randomisation procedures have been described in detail [18].

The study was approved by the Ethics Committee at Göteborg University, Sweden. All participants gave written informed consent at entry.

2.2. Anthropometric measurements

Height was measured to the nearest 0.5 cm and weight registered to the nearest 0.1 kg. Waist and hip circumference were measured according to WHO [19]. Sagittal diameter was measured to the nearest 0.1 cm in the recumbent position midway between the inferior margin of the last rib and the crest of the ileum in the sagittal plane.

2.2.1. Body composition

Bioelectrical impedance (single frequency, 50 kHz) (Animeter, HTS, Odense, Denmark) was measured in the fasting state. Calculation of lean body mass and percentage body fat was performed according to Segal et al. [20].

2.3. Lifestyle intervention

2.3.1. Control group

After baseline examination and randomisation, this group received a letter informing them that they

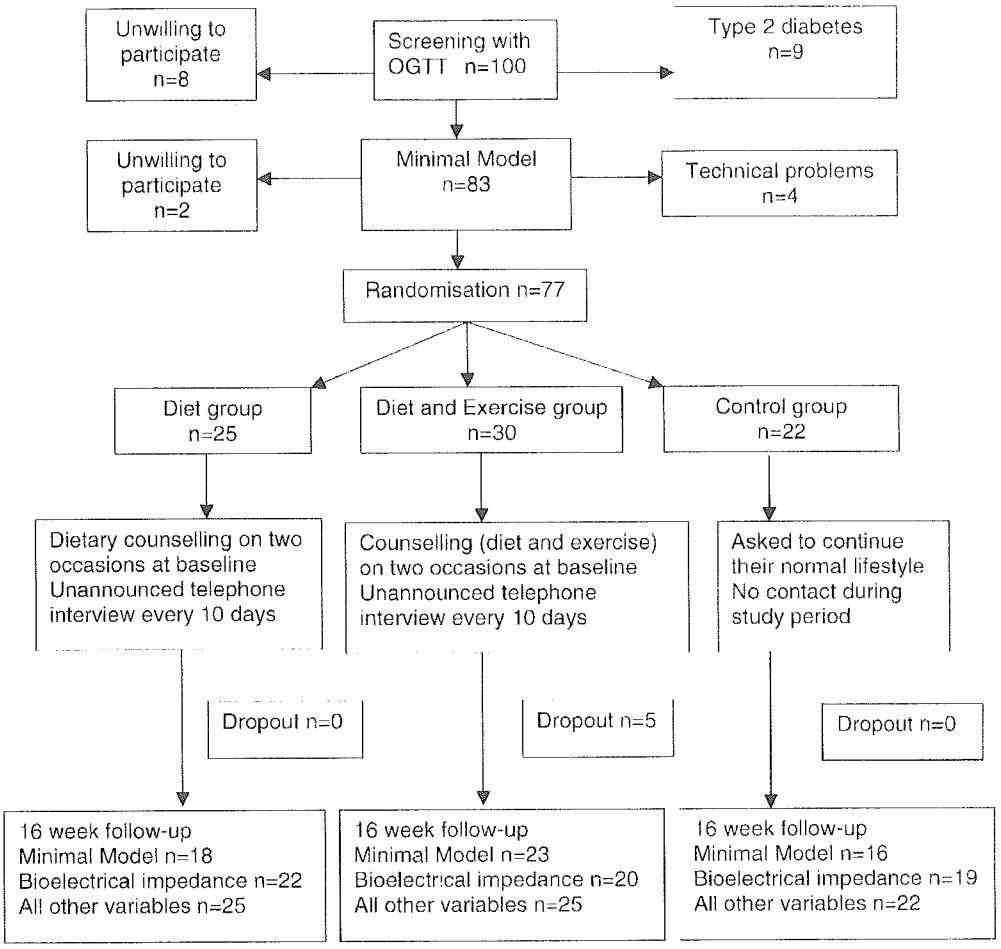

Fig. 1. Flow diagram over screening, inclusion and intervention in first-degree relatives of type 2 diabetic patients: OGTT: oral glucose tolerance test.

should continue with their current lifestyle and receive lifestyle intervention 1 year later.

2.3.2. *Intervention groups*

Dietary counselling, given on two occasions, was based on current nutrition recommendations ($\leq$30% of energy (*E*%) from fat, $\leq$10*E*% from saturated fat, $\geq$12 g fiber/1000 kcal) [21], including increased intake of fatty fish and low glycaemic index foods. The goal for exercise (DE-group) was to increase physical activity by walking or more intensive activities for at least 30 min, 4–5 times per week, regardless of present activity level. Intensive follow-up was performed through telephone interviews, without prior notification, every 10 days including a 24 h dietary recall and a 72 h physical activity recall. Adherence to dietary and exercise advice was monitored by a semi-quantitative food frequency questionnaire (FFQ) [22] and leisure time physical activity interview [23,24] at baseline and after 16 weeks.

2.4. *Subgroup analysis*

A subgroup analysis was performed to study the metabolic effects of intervention in FDR with the best concordance with dietary goals and increased physical activity, independent of treatment group. In short, the mean nutrient composition of eight dietary recalls from each person was calculated and presented as percent of energy. Each individual was given a ranking number (1–50) depending on the outcome of five dietary factors (saturated fat, very long chain (VLC) $n-3$, 18:3, $n-3$, fibre, average daily glycaemic index

(GI)) and each factor was given equal "weight". The sum of ranking numbers was calculated for every FDR and those in the upper half considered concordant. Based on physical activity interviews, the FDR were divided into two groups — increased physical activity and no change or decreased physical activity. The FDR adherent to both dietary advice and increased physical activity constituted the DE+-group.

2.5. Laboratory procedures

Subjects were asked to abstain from food and alcohol intake and use of nicotine after 9.00 p.m. on the preceding evening and to eat as "normal" as possible and not to perform strenuous exercise during the 2 days preceding examination.

2.5.1. Assessment of insulin sensitivity

Bergmans minimal model was performed at baseline and after 16 weeks. Glucose 30%, 0.3 g/kg BW, was given iv via an antecubital vein while frequent blood samples were drawn from the contralateral vein. Twenty minutes after glucose injection, insulin was administered iv (0.03 U/kg BW) and venous blood sampling continued until 180 min. Glucose and insulin were analysed at 20 time points: −5, −1, 2, 4, 6, 8, 10, 14, 19, 22, 30, 40, 50, 60, 70, 90, 100, 120, 140 and 180 min, and insulin sensitivity index (S_I) was calculated [25,26].

2.5.2. Biochemical measurements

Blood glucose was analysed immediately using an automatic glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, USA) while plasma and serum were frozen (−20 or −70 °C) for later analysis. Plasma insulin was analysed by a radioimmunoassay (Pharmacia, Uppsala, Sweden). Total cholesterol, triglycerides and HDL cholesterol were analysed by standard methods at the Central Laboratory Sahlgrenska University Hospital and LDL cholesterol was calculated from Friedewalds formula [27]. LDL size was measured with gradient gel electrophoresis [28]. Apolipoprotein B concentration was measured by immunoturbidometric Cobas Fara II autoanalysers (Unimate 2 ApoA/ApoB, Hoffmann-La Roche, Basel, Switzerland). Apo E genotype was determined by a polymerase chain reaction technique. The determination of cotinine was performed by capillary gas chromatography after a single step liquid–liquid extraction of the plasma samples. Leptin was measured with a radioimmunoassay (Linco Research, St Louis, USA). Adiponectin was analysed by an ELISA (Otsuka Pharmaceutical Co.) technique [29]. All paired samples from one individual were analysed in the same batch.

As an independent measure of consumption of dietary fatty acids, fatty acid composition of erythrocyte membranes was analysed. Isolation of red blood cells and lipid extraction was done essentially as described by Nilsson et al. [30]. Alkaline transmethylation of the erythrocyte glycerophospholipids, isolation of the formed fatty acid methyl esters and analysis by capillary gas chromatography was performed according to Landen et al. [31].

2.6. Statistical analysis

Sample size calculation ($n = 75$, 25 subjects in each treatment group) was based on detecting a 20% improvement in insulin sensitivity at 80% power. Baseline data are presented as mean (S.D.) and mean changes with 95% confidence interval (CI). One-way ANOVA (with Tukey's test as post-hoc) was used for comparisons of changes between groups. Two-sample t-test was used to compare changes in the DE+- and C-groups. A P-value less than 0.05 was considered statistically significant. Pearson's correlation coefficients were used to study co-variation between variables and a P-value of 0.01 was considered statistically significant. Spearman's ρ was used to study co-variation in the subgroup (DE+). SPSS for Windows (version 10.0, 1999, Chicago, IL) was used for all statistical analyses.

3. Results

3.1. Baseline characteristics

Results are shown for the 72 FDR who completed the 16-week study. Baseline values for anthropometry and biochemical measurements were similar in the three groups (Table 1). Average levels of biochemical variables were all within normal values for a healthy population. In addition, Apo E genotypes were evenly distributed among the groups (data not shown). Baseline dietary composition [18] was similar to the

Table 1
Participant characteristics at baseline

	D-group	DE-group	C-group
n (female/male)	25 (10/15)	25 (7/18)	22 (9/13)
IFG/IGT, *n*	4/0	3/1	2/0
Nicotine users, *n*	9	12	9
Age (year)	43.7 (7.5)	41.8 (9.2)	42.1 (8.7)
BMI (kg/m^2)	25.3 (3.6)	26.0 (3.4)	26.0 (2.7)
Cotinine (ng/ml)[a]	290 (150)	268 (165)	254 (168)
Body weight (kg)	79.2 (12.9)	79.2 (10.4)	78.3 (11.5)
Body fat (%)	28.0 (7.8) (*n* = 22)	29.5 (7.4) (*n* = 20)	29.3 (7.0) (*n* = 19)
Waist circumference (cm)	89.2 (10.7)	91.2 (9.4)	90.2 (10.8)
Sagittal diameter (cm)	20.1 (2.9)	20.8 (3.0)	20.7 (2.7)
Systolic blood pressure (mmHg)	113 (14)	117 (12)	117 (16)
Diastolic blood pressure (mmHg)	69 (7)	74 (9)	69 (11)
Fasting blood glucose (mmol/l)	4.6 (0.5)	4.7 (0.4)	4.6 (0.5)
Fasting insulin (mU/l)	7.6 (3.0)	9.3 (4.7)	8.5 (4.5)
S_i ($\times 10^{-4}$ min^{-1} per mU/l)	3.50 (1.73) (*n* = 18)	3.17 (2.55) (*n* = 23)	3.80 (1.62) (*n* = 16)
Total cholesterol (mmol/l)	4.82 (0.71)	4.82 (0.82)	4.95 (1.09)
HDL cholesterol (mmol/l)	1.27 (0.27)	1.17 (0.27)	1.31 (0.27)
LDL cholesterol (mmol/l)	3.02 (0.61)	3.06 (0.72)	3.16 (0.97)
LDL particle size (nm)	26.4 (1.1)	26.0 (1.2)	26.4 (1.1)
Apolipoprotein B (mg/dl)	91.4 (20.5)	95.3 (25.0)	91.2 (28.5)
Triglycerides (mmol/l)	1.17 (0.49)	1.32 (0.69)	1.07 (0.50)
Adiponectin (μg/ml)	7.04 (3.27)	5.70 (3.08)	6.03 (2.05)
Leptin (ng/ml)	10.0 (9.3)	9.5 (8.2)	10.0 (7.6)

Data are presented as mean (S.D.). D-group: diet group; DE-group: diet and exercise group; C-group: control group; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; BMI: body mass index; S_i: insulin sensitivity index.

[a] Cotinin level in nicotine users.

general Swedish diet [32]. Thus, the intake of total and saturated fat was higher and dietary fiber lower than recommended [21].

3.2. Adherence to lifestyle intervention

According to food frequency questionnaires, both intervention groups decreased intake of saturated fatty acids (−4% of total energy intake, $P < 0.001$), increased intake of dietary fibre (+4 g/1000 kcal, $P \leq 0.001$) and lowered average GI (−4 to −6 U, $P < 0.01$) compared to controls. Group D increased their intake of α-linolenic acid (18:3, $n-3$) significantly (+0.3% of energy, $P < 0.01$ versus controls), while intake of very long chain $n-3$ fatty acids from fish increased (+0.2% of energy, $P < 0.000$ versus controls) in both groups.

Changes in fatty acid composition of the erythrocyte membranes in the D- and DE-groups are shown in Fig. 2. An increase in the proportion of the very long chain $n-3$ fatty acid 22:6 was accompanied by a corresponding reduction in the proportion of the $n-6$ fatty acids 20:3, 20:4, 22:4 and 22:5 in both groups when compared to the C-group. Although there was no significant difference in change in fatty acid composition between the treatment groups, a trend for better compliance to dietary advice regarding quality of fat was observed in the D-group.

Median physical activity (min/week) did not change in any group although an increase was seen in the least active subjects in DE-group (+70 min/week, $P < 0.01$ within-group).

3.3. Effects of lifestyle intervention

The DE-group reduced body weight, waist circumference and sagittal diameter (Table 2). A 13% reduction in fasting insulin was observed within the DE-group, the D- and DE-groups, however, showed no significant change in fasting glucose, insulin or S_i when compared to the C-group. The D-group reduced total cholesterol (−0.31 mmol/l), LDL cholesterol

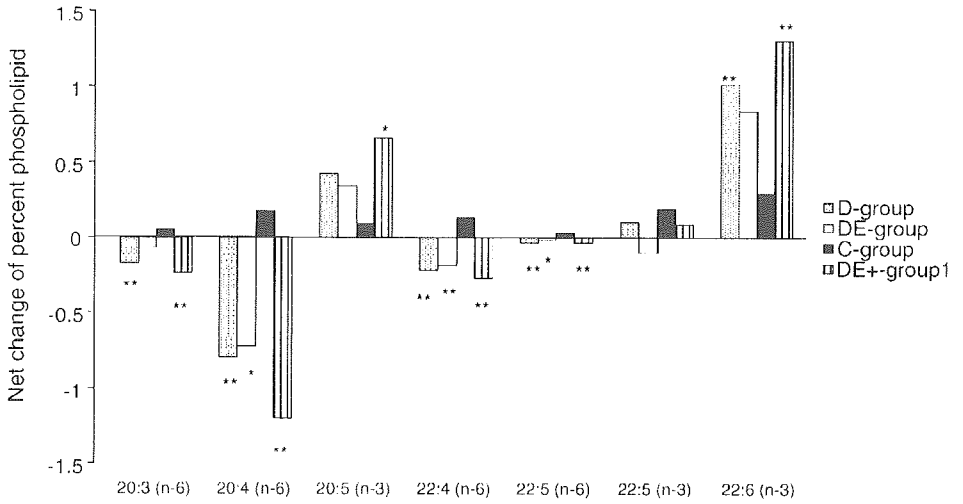

Fig. 2. Change in $n-3$ and $n-6$ fatty acids of the erythrocyte membrane after 16 weeks intervention. D-group: diet group; DE-group: diet and exercise group; C-group: control group; DE+-group: 13 subjects with best adherence to dietary advice and increased exercise; $^{*}P < 0.05$ vs. C-group; $^{**}P < 0.01$ vs. C-group.

(−0.22 mmol/l) and apolipoprotein B (−9.5 mg/dl), and the decrease remained significant after correcting for change in body weight. The within-group significant decrease in circulating leptin levels in the D-group was of borderline significance compared to the C-group. In addition, circulating adiponectin increased within the DE-group but did not change in the two other groups (Table 2).

Thirteen individuals (11 men, 2 women) were considered concordant with dietary goals and increased their physical activity independent of treatment group (D or DE). As shown in Fig. 2, this group showed the greatest change in fatty acid composition of erythrocyte membranes, confirming their effort to make dietary adjustments. Group mean characteristics and changes are described in Table 3. An increased S_i, as well as reduced total cholesterol, triglycerides, LDL cholesterol, apolipoprotein B, waist circumference and sagittal diameter was shown, whereas circulating leptin and adiponectin were unaltered throughout the intervention.

3.4. Correlation analysis

Minimal model tests could be performed in 57 FDR before and after intervention. The scattered changes that occurred in S_i in these individuals, significantly ($P < 0.01$) correlated to change in plasma fasting insulin ($r = -0.52$), total cholesterol ($r = -0.51$), LDL cholesterol ($r = -0.53$), LDL size ($r = 0.47$), apolipoprotein B ($r = -0.61$), triglycerides ($r = -0.52$), body fat ($r = -0.42$) and dihomo-γ-linolenic acid (20:3, $n-6$) in erythrocyte membranes ($r = -0.38$). Change in circulating adiponectin showed no correlation with change in S_i. In the subgroup (DE+), where S_i was improved, associations were observed between change in S_i and change in blood glucose ($r_s = -0.84$) and apo B ($r_s = -0.78$) ($P < 0.01$).

4. Discussion

Our implementation of a multifactorial lifestyle intervention showed that significant improvements can be achieved in metabolic variables in "healthy" FDR of type 2 diabetic subjects. The most pronounced effects were observed in the D-group with clear reductions in blood lipid variables. In the DE-group positive changes occurred in body weight and body fat distribution with a tendency to reduced fasting insulin. In those individuals making the strongest effort regarding dietary adherence and increased physical activity, improvements were also seen in insulin sensitivity.

The salient findings on lipid variables were noticed in the D-group where total cholesterol, LDL cholesterol and apolipoprotein B were decreased by 6, 7 and 10%, respectively. These reductions are

Table 2
Changes after 16 weeks intervention, mean (95% CI)

	ΔD-group (n = 25)	ΔDE-group (n = 25)	ΔC-group (n = 22)	P-value		
				D vs. DE	D vs. C	DE vs. C
Nicotine users, n	−1	−1	0	–	–	–
Cotinine (ng/ml)	−8 (−46, 30)	−11 (−61, 39)	−4 (−49, 40)	0.994	0.993	0.970
Body weight (kg)	−0.8 (−1.8, 0.1)	−1.7 (−2.8, −0.5)	0.5 (−1.0, 1.9)	0.541	0.261	0.030
Body fat (%)	−0.3 (−0.9, 0.3) (n = 22)	−0.9 (−2.0, 0.2) (n = 20)	0.01 (−0.7, 0.7) (n = 19)	0.461	0.887	0.250
Waist circumference (cm)	−1.0 (−2.0, 0.1)	−3.0 (−4.6, −1.4)	1.2 (−0.4, 2.7)	0.088	0.081	0.000
Sagittal diameter (cm)	−1.0 (−1.5, −0.5)	−1.3 (−1.9, −0.8)	−0.1 (−0.7, 0.5)	0.643	0.057	0.006
Fasting blood glucose (mmol/l)	−0.11 (−0.28, 0.06)	−0.13 (−0.31, 0.05)	−0.13 (−0.29, 0.02)	0.998	0.986	1.000
Fasting insulin (mU/l)	0.5 (−1.6, 0.6)	−1.2 (−2.3, −0.2)	0.1 (−1.4, 1.7)	0.628	0.740	0.238
S_i ($\times 10^{-4}$ min^{-1} per mU/l)	0.70 (−0.05, 1.45) (n = 18)	0.27 (−0.33, 0.87) (n = 23)	0.10 (−0.74, 0.54) (n = 16)	0.588	0.220	0.690
Total cholesterol (mmol/l)	−0.31 (−0.57, −0.05)	0.11(−0.12, 0.33)	0.15 (−0.11, 0.42)	0.039	0.024	0.960
HDL cholesterol (mmol/l)	−0.026 (−0.10, 0.05)	0.019 (−0.35, 0.74)	−0.033 (−0.08, 0.01)	0.515	0.988	0.448
LDL cholesterol (mmol/l)	−0.22 (−0.45, 0.02)	0.12 (−0.07, 0.31)	0.19 (−0.03, 0.41)	0.058	0.021	0.876
LDL particle size (nm)	0.097 (−0.13, 0.33)	0.048 (−0.19, 0.28)	0.066 (−0.28, 0.41)	0.959	0.985	0.994
Apo B (mg/dl)	9.5 (−14.8, −4.1)	−0.9 (−6.9, 5.2)	2.9 (−3.4, 9.2)	0.082	0.009	0.624
Triglycerides (mmol/l)	−0.13 (−0.26, 0.001)	−0.06 (−0.22, 0.09)	0.006 (−0.16, 0.15)	0.761	0.435	0.843
Adiponectin (μg/ml)	0.38 (−0.93, 0.17)	0.63 (0.17, 1.09)	0.06 (−0.39, 0.26)	0.005	0.590	0.087
Leptin (ng/ml)	1.5 (−2.9, −0.1)	−0.9 (−2.1, 0.2)	0.5 (−0.7, 1.7)	0.781	0.054	0.208

D-group: diet group; DE-group: diet and exercise group; C-group: control group; S_i: insulin sensitivity index.

Table 3
Post-hoc analysis of 13 individuals with best adherence to dietary advice and increased exercise (DE+)

	Baseline DE+ (n = 13)	ΔDE+ (n = 13)	DE+ vs. C, P-value
Body weight (kg)	78.6 (8.4)	−1.8 (−3.6, 0.0)	0.050
Body fat (%)	24.4 (6.8) (n = 11)	−1.0 (−2.2, 0.3)	0.138
Waist circumference (cm)	90.0 (9.7)	−2.7 (−4.6, −0.8)	0.003
Sagittal diameter (cm)	20.3 (3.0)	−1.5 (−2.4, −0.7)	0.007
Fasting blood glucose (mmol/l)	4.8 (0.4)	−0.2 (−0.5, 0.0)	0.530
Fasting insulin (mU/l)	9.00 (5.04)	−1.21 (−2.80, 0.38)	0.233
S_i ($\times 10^{-4}$ min^{-1} per mU/l)	3.27 (1.46) (n = 10)	1.43 (0.17, 2.68)	0.010
Total cholesterol (mmol/l)	5.16 (0.81)	−0.36 (−0.68, −0.04)	0.014
HDL cholesterol (mmol/l)	1.16 (0.30)	0.02 (−0.07, 0.10)	0.279
LDL cholesterol (mmol/l)	3.41 (0.64)	−0.25 (−0.52, 0.02)	0.012
LDL particle size (nm)	25.9 (1.17)	−0.20 (−0.12, 0.53)	0.580
Apolipoprotein B (mg/dl)	101.8 (26.2)	−12.4 (−19.8, −5.0)	0.003
Triglycerides (mmol/l)	1.31 (0.63)	−0.28 (−0.48, −0.09)	0.024
Adiponectin (μg/ml)	5.9 (2.7)	0.04 (−0.63, 0.55)	0.942
Leptin (ng/ml)	7.0 (5.1)	−0.5 (−1.8, 0.8)	0.241

Data are expressed as mean (S.D.) and change with 95% CI; DE+: 11 males and 2 females (mean age: 42.6 (9.7) years), 7 subjects from diet group and 6 subjects from diet and exercise group; S_i: insulin sensitivity index.

comparable to or exceed those observed in earlier lifestyle intervention studies [11,33–35] where average baseline LDL cholesterol was 4.23 mmol/l [33–35], i.e. 37% higher than in the present study. LDL cholesterol is a strong predictor of coronary heart disease in diabetic individuals, even at concentrations below the target of the National Cholesterol Education Program of 3.4 mmol/l [36]. As a consequence, our results may be clinically important in the light of the increased risk of atherosclerosis in FDR [8]. Interestingly, the positive findings on blood lipid variables were independent of change in body weight. This indicates that it is possible to achieve reductions in risk factors for atherosclerosis without focusing on the often so "unattainable" body weight reduction.

Although weight reduction was not a primary goal of this intervention, the DE-group decreased their body weight by 2.1%. Significant decreases were also seen in waist circumference and sagittal abdominal diameter, indicating improved body fat distribution, which may lead to reduced cardiovascular risk [37]. Also, within the DE-group, we observed a 13% reduction in fasting insulin, in concordance with their weight reduction. The relative size of this reduction is similar to the 1-year results from The Finnish diabetes prevention study [11], although their baseline level was 60% higher. It is also comparable to changes observed in the DE-group of The Oslo Diet and Exercise Study (ODES) [38] where exercise was specifically emphasized. Although we did not observe significant changes in S_i in any group, the reduced within-group fasting insulin might indicate reduction in insulin resistance [39].

Our results may indicate that diet treatment yields effects different from treatment with diet and exercise. However, the observed change in fatty acid composition of the erythrocyte membrane suggests that the D-group made stronger efforts than the DE-group regarding change in dietary fat quality. Canola oil, rich in α-linolenic acid, reduces LDL cholesterol when substituted for fats in an average Dutch diet [40]. The subgroup (DE+), derived from both D-group and DE-group showed even greater changes in erythrocyte membrane composition. In this group, the diet was characterized by a predominant use of low-fat products of milk, cheese and margarine. High fibre bread, fruit and vegetables were used daily, and fatty fish at least three times/week. The median increase in time spent for physical activity was 80 min/week. As DE+-group showed improvements in S_i, body fat distribution and most lipids, this suggests that the optimal treatment is a combination of diet and exercise. However, dietary counselling alone also lead to a significant reduction in important risk factors for coronary heart disease.

Apolipoprotein B has previously been reported to be elevated by 13% in FDR compared to control subjects [8]. We observed reductions of 10% (D-

group) and 12% (DE+-group) in Apo B level after 16 weeks of lifestyle intervention. Previous studies have linked Apo B to serum insulin concentrations [8,41]. Further, we noticed a strong inverse correlation between change in Apo B and change in S_i in all subjects, supporting a link between insulin resistance and Apo B.

In subjects characterised by the metabolic syndrome, long-term (1 year) lifestyle intervention has been reported to reduce plasma leptin beyond the expected reduction due to decreased fat mass [42]. In our short-term study, we also observed a trend towards decreased leptin within the D-group. This finding should be added to the known importance of keeping the leptin levels low to minimise cardiovascular risk [43].

The actual changes in adiponectin levels in this study were trivial, but we noticed a trend for increased adiponectin in the DE-group where body weight and abdominal obesity decreased. This is in line with studies involving large body weight reductions (>10% of body weight) [44,45]. However, this is to our knowledge the first attempt to address whether lifestyle intervention not aiming at weight reduction is linked to change in adiponectin. Clearly more studies are needed to clarify this matter in FDR.

A 16-week study period was long enough to show the effectiveness of the dietary as well as dietary and exercise treatment program on several of the measured variables linked to cardiovascular risk, but feasibility of the treatments should be evaluated in long-term studies. Unfortunately, due to technical problems, we could not repeat the minimal model in 15 subjects and did not achieve enough statistical power to address the effect on S_i in the primary analysis. We did observe an increased S_i in the subgroup analysis. However, it should be noted that this analysis was not identified a priori. The finding could be due to the increased risk of statistical error and/or chance. Moreover, our results might be hampered by the fact that the intervention groups included subjects who were related to each other. However, they were allocated in the same groups to avoid treatment confounding.

In conclusion, this lifestyle intervention study shows that even "healthy" first-degree relatives of patients with type 2 diabetes can significantly improve risk factors associated with insulin resistance and cardiovascular disease.

Acknowledgements

The financial support was given by The Regional Health Care Authority of West Sweden, The Swedish Diabetes Association, The Inga-Britt and Arne Lundberg Foundation, the Novo Nordisk Foundation, Helsinki University Central Hospital Research Foundation and The Sigrid Jusélius Foundation. The valuable comments on the study design given by Professor Ulf Smith, Dr. Bengt Persson, Dr. Annika Adlerberth, Dr. Ali Taghavi, Dr. Birgitta Svensson, Dr. Staffan Wettre and Dr. Lotte Waller are gratefully acknowledged. We thank Åsa Sunesson and Else H. Johanson for help with telephone interviews and counselling. We also thank Margareta Landen, Lotta Kaire, Eva Alfvegren, Birgitta Jungbjer and Helinä Perttunen-Nio for technical assistance.

References

[1] J. Köbberling, H. Tillil, Empirical risk figures for first-degree relatives of non-insulin dependent diabetics, in: J. Köbberling, R. Tattersall (Eds.), The Genetics of Diabetes Mellitus, Academic Press, London and New York, 1982, pp. 201–209.

[2] S.P. O'Rahilly, Z. Nugent, A.S. Rudenski, et al. Beta-cell dysfunction, rather than insulin insensitivity, is the primary defect in familial type 2 diabetes, Lancet 2 (1986) 360–364.

[3] J. Eriksson, A. Franssila-Kallunki, A. Ekstrand, et al. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus, N. Engl. J. Med. 321 (1989) 337–343.

[4] W. Pimenta, M. Korytkowski, A. Mitrakou, et al. Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative, JAMA 273 (1995) 1855–1861.

[5] G. Perseghin, S. Ghosh, K. Gerow, G.I. Shulman, Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study, Diabetes 46 (1997) 1001–1009.

[6] J.E. Henriksen, K. Levin, P. Thye-Ronn, et al. Glucose-mediated glucose disposal in insulin-resistant normoglycaemic relatives of type 2 diabetic patients, Diabetes 49 (2000) 1209–1218.

[7] S.M. Haffner, M.P. Stern, H.P. Hazuda, B.D. Mitchell, J.K. Patterson, E. Ferrannini, Parental history of diabetes is associated with increased cardiovascular risk factors, Arteriosclerosis 9 (1989) 928–933.

[8] M.W. Stewart, D.B. Humphriss, J. Mitcheson, J. Webster, M. Walker, M.F. Laker, Lipoprotein composition and serum apolipoproteins in normoglycaemic first-degree relatives of non-insulin dependent diabetic patients, Atherosclerosis 139 (1998) 115–121.

[9] B. Nyholm, S. Fisker, S. Lund, N. Moller, O. Schmitz, Increased circulating leptin concentrations in insulin-resistant first-degree relatives of patients with non-insulin-dependent diabetes mellitus: relationship to body composition and insulin sensitivity but not to family history of non-insulin-dependent diabetes mellitus, Eur. J. Endocrinol. 136 (1997) 173–179.
[10] F. Pellme, U. Smith, T. Funahashi, et al. Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients, Diabetes 52 (2003) 1182–1186.
[11] J. Tuomilehto, J. Lindstrom, J.G. Eriksson, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance, N. Engl. J. Med. 344 (2001) 1343–1350.
[12] W.C. Knowler, E. Barrett-Connor, S.E. Fowler, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin, N. Engl. J. Med. 346 (2002) 393–403.
[13] A.J. Adamson, E. Foster, T.J. Butler, S. Bennet, M. Walker, Non-diabetic relatives of type 2 diabetic families: dietary intake contributes to the increased risk of diabetes, Diabetes Med. 18 (2001) 984–990.
[14] B. Vessby, Dietary fat and insulin action in humans, Br. J. Nutr. 83 (Suppl. 1) (2000) S91–S96.
[15] F.M. Sacks, M. Katan, Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease, Am. J. Med. 113 (Suppl. 9B) (2002) 13S–24S.
[16] K.G. Alberti, P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, Diabetes Med. 15 (1998) 539–553.
[17] D.G. Altman, Clinical trials, in: D.G. Altman (Ed.), Practical Statistics in Medical Research, Chapman & Hall, London, 1991, pp. 440–474.
[18] H.K. Brekke, P.A. Jansson, J.E. Mansson, R.A. Lenner, Lifestyle changes can be achieved through counselling and follow-up in first-degree relatives of patients with type 2 diabetes, J. Am. Diet Assoc. 103 (2003) 835–843.
[19] World Health Organisation, Physical Status: The Use and Interpretation of Anthropometry, WHO, Geneva, 1985, pp. 430–431.
[20] K.R. Segal, M. Van Loan, P.I. Fitzgerald, J.A. Hodgdon, T.B. Van Itallie, Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study, Am. J. Clin. Nutr. 47 (1988) 7–14.
[21] Nordic Council of Ministers, Nordic Nutrition Recommendations, Nord, Copenhagen, 1996.
[22] I. Johansson, G. Hallmans, A. Wikman, C. Biessy, E. Riboli, R. Kaaks, Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort, Publ. Health Nutr. 5 (2002) 487–496.
[23] H.L. Taylor, D.R. Jacobs Jr., B. Schucker, J. Knudsen, A.S. Leon, G. Debacker, A questionnaire for the assessment of leisure time physical activities, J. Chronic Dis. 31 (1978) 741–755.
[24] M.T. Richardson, A.S. Leon, D.R. Jacobs Jr., B.E. Ainsworth, R. Serfass, Comprehensive evaluation of the Minnesota Leisure Time Physical Activity Questionnaire, J. Clin. Epidemiol. 47 (1994) 271–281.
[25] R.N. Bergman, Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach, Diabetes 38 (1989) 1512–1527.
[26] G.M. Steil, A. Volund, S.E. Kahn, R.N. Bergman, Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Suitability for use in population studies, Diabetes 42 (1993) 250–256.
[27] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[28] J. Vakkilainen, M. Jauhiainen, K. Ylitalo, et al. LDL particle size in familial combined hyperlipidemia: effects of serum lipids, lipoprotein-modifying enzymes, and lipid transfer proteins, J. Lipid Res. 43 (2002) 598–603.
[29] Y. Arita, S. Kihara, N. Ouchi, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity, Biochem. Biophys. Res. Commun. 257 (1999) 79–83.
[30] O. Nilsson, G. Hakansson, S. Dreborg, C.G. Groth, L. Svennerholm, Increased cerebroside concentration in plasma and erythrocytes in Gaucher disease: significant differences between type I and type III, Clin. Genet. 22 (1982) 274–279.
[31] M. Landen, P. Davidsson, C.-G. Gottfries, J.-E. Månsson, K. Blennow, Reduction in the synaptophysin level but normal levels of glycerophospholipids in the gyrus cinguli in schizophrenia, Schizophr. Res. 55 (2002) 83–88.
[32] Livsmedelsverket, Riksmaten, Dietary habits and nutrient intake in Sweden 1997–98. www.livsmedelsverket.se/Mat&Hälsa/Nutrition/Matvanor/Vuxna.
[33] M.L. Hellenius, U. de Faire, B. Berglund, A. Hamsten, I. Krakau, Diet and exercise are equally effective in reducing risk for cardiovascular disease. Results of a randomized controlled study in men with slightly to moderately raised cardiovascular risk factors, Atherosclerosis 103 (1993) 81–91.
[34] D.M. Bourn, J.I. Mann, B.J. McSkimming, M.A. Waldron, J.D. Wishart, Impaired glucose tolerance and NIDDM: does a lifestyle intervention program have an effect? Diabetes Care 17 (1994) 1311–1319.
[35] K.A. McAuley, S.M. Williams, J.I. Mann, et al. Intensive lifestyle changes are necessary to improve insulin sensitivity: a randomized controlled trial, Diabetes Care 25 (2002) 445–452.
[36] B.V. Howard, D.C. Robbins, M.L. Sievers, et al. LDL cholesterol as a strong predictor of coronary heart disease in diabetic individuals with insulin resistance and low LDL: the strong heart study, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 830–835.
[37] M. Ohrvall, L. Berglund, B. Vessby, Sagittal abdominal diameter compared with other anthropometric measurements in relation to cardiovascular risk, Int. J. Obest. Relat. Metab. Disord. 24 (2000) 497–501.

[38] P.A. Torjesen, K.I. Birkeland, S.A. Anderssen, I. Hjermann, I. Holme, P. Urdal, Lifestyle changes may reverse development of the insulin resistance syndrome. The Oslo Diet and Exercise Study: a randomized trial, Diabetes Care 20 (1997) 26–31.

[39] M. Laakso, How good a marker is insulin level for insulin resistance? Am. J. Epidemiol. 137 (1993) 959–965.

[40] M.B. Katan, P.L. Zock, R.P. Mensink, Dietary oils, serum lipoproteins, and coronary heart disease, Am. J. Clin. Nutr. 61 (1995) 1368S–1373S.

[41] P.H. Winocour, S. Kaluvya, K. Ramaiya, et al. Relation between insulinemia, body mass index, and lipoprotein composition in healthy, nondiabetic men and women, Arterioscler. Thromb. 12 (1992) 393–402.

[42] J.E. Reseland, S.A. Anderssen, K. Solvoll, et al. Effect of long-term changes in diet and exercise on plasma leptin concentrations, Am. J. Clin. Nutr. 73 (2001) 240–245.

[43] F. Leyva, I.F. Godsland, M. Ghatei, et al. Hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 928–933.

[44] K. Hotta, T. Funahashi, Y. Arita, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1595–1599.

[45] W.S. Yang, W.J. Lee, T. Funahashi, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin, J. Clin. Endocrinol. Metab. 86 (2001) 3815–3819.

Circ J 2005; **69:** 1154–1156

Hypoadiponectinemia is Associated With Coronary Artery Spasm in Men

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Background The relationship between adiponectin and coronary spastic angina (CSA), both of which are closely involved in coronary endothelial dysfunction, has not been elucidated.
Methods and Results Plasma adiponectin concentrations were examined in 55 men with CSA and 55 with chest pain syndrome (CPS). The plasma log-adiponectin levels were significantly lower in patients with CSA than with CPS (0.61±0.28 vs 0.80±0.21 μg/ml, p<0.0001). The prevalence of smoking was significantly higher in the CSA patients than in those with CPS (50.9% vs 29.1%, p=0.0195). In multiple logistic regression analysis, log-adiponectin (p=0.0008) and smoking (p=0.0210) were independent determinants of CSA.
Conclusions Hypoadiponectinemia is a potential risk factor for CSA in men, independent of smoking. (*Circ J* 2005; **69:** 1154–1156)

Key Words: Adiponectin; Coronary artery spasm; Smoking

Chronic coronary artery disease (CAD) is most commonly the result of obstruction of the coronary arteries by atheromatous plaques. However, coronary artery spasm, which is defined as an abnormal contraction of an epicardial coronary artery that results in myocardial ischemia, is also often observed and plays an important role in the pathogenesis of ischemic heart disease in Japan[1] Endothelial-derived nitric oxide (NO) is a key determinant of coronary artery spasm, and is synthesized by endothelial NO synthase (eNOS)[1]

Adiponectin has a protective action against the initiation and progression of atherosclerosis through its anti-inflammatory and anti-atherosclerotic effects[2,3] In cultured endothelial cells adiponectin also ameliorates the suppression of eNOS activity, leading to increased NO production[2] Patients with CAD have low levels of adiponectin[2] and clinically, low plasma levels of adiponectin have been also reported to be associated with vascular endothelial dysfunction in humans[4] However, the relationship between adiponectin and coronary artery spasm, both of which are closely involved in coronary endothelial dysfunction, has not been elucidated and so the hypothesis of the present study was that adiponectin may be associated with coronary spastic angina (CSA).

(Received May 24, 2005; revised manuscript received July 11, 2005; accepted July 19, 2005)
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Methods

We enrolled 110 Japanese men who underwent diagnostic cardiac catheterization at Kumamoto University Hospital (age: 63.6±9.9 years, mean±SD, range: 34–82 years). The group with CSA comprised 55 consecutive patients who fulfilled both inclusion criteria: (a) spontaneous anginal attacks associated with ST-segment elevation or depression on the ECG at rest; and (b) coronary artery spasm demonstrated angiographically by intracoronary infusion of acetylcholine (ACh)[5] The chest pain syndrome (CPS) group comprised 55 subjects in whom coronary artery spasm could not be provoked in any coronary artery by intracoronary infusion of ACh. The CPS group was matched for age and body mass index (BMI) with the CSA group. Incremental doses of ACh were infused into the left and right coronary arteries separately as described previously[5] Coronary artery spasm was defined as brief total or subtotal occlusion of the epicardial coronary arteries associated with chest pain and/or ischemic ST-segment changes on the electrocardiogram. Neither group had ≥25% organic coronary artery stenosis and none of the patients were taking any type of thiazolidinediones or calcium antagonists. Plasma adiponectin levels were determined by an enzyme-linked immunosorbent assay as described previously[6]

All data are expressed as mean±SD. Differences in frequencies were analyzed by the chi-square test. Differences in continuous variables between 2 groups were examined using an unpaired t-test. Because the distribution of the levels of plasma adiponectin, serum triglyceride, and C-reactive protein were skewed, logarithmically transformed values were used for statistical analysis. To examine the independent factors for CSA, multiple logistic regression analysis was performed including the variables that were significant in simple logistic regression analysis. A p-value <0.05 was considered statistically significant.

Table 1 Clinical and Biochemical Characteristics of the 2 Study Groups

	Coronary spastic angina (n=55)	Chest pain syndrome (n=55)	p value
Age, years	63.5±9.8	63.6±10.1	0.9847
Hypertension, n (%)	19 (34.5)	27 (49.1)	0.2461
Systolic blood pressure, mmHg	127±18	130±18	0.4137
Diastolic blood pressure, mmHg	79±10	77±11	0.4088
Pulse rate, beats/min	66±10	66±10	0.9123
Diabetes mellitus, n (%)	12 (21.8)	18 (32.7)	0.0868
Cigarette smoking, n (%)	28 (50.9)	16 (29.1)	0.0195
Fasting blood glucose, mg/dl	92.0±23.7	95.1±18.6	0.4491
HbA1c, %	5.5±1.3	5.9±1.1	0.0999
TC, mg/dl	189.4±31.2	192.4±33.6	0.7447
Log-TG, mg/dl	2.10±0.21	2.07±0.20	0.5576
HDL-C, mg/dl	48.8±13.7	55.7±20.2	0.0403
LDL-C, mg/dl	121.7±27.6	118.1±27.1	0.4998
Fibrinogen, mg/dl	324.7±75.7	308.7±63.6	0.2413
BMI, kg/m^2	23.8±2.96	23.8±3.08	0.9222
Creatinine, mg/dl	0.87±0.19	0.91±0.24	0.2396
Log-CRP, mg/dl	−1.10±0.35	−1.09±0.41	0.8974
Log-adiponectin, μg/ml	0.61±0.28	0.80±0.21	<0.0001
Family history of CAD, n (%)	14 (25.5)	10 (18.2)	0.4886

HbA1c, hemoglobin A1c; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index; CRP, C-reactive protein; CAD, coronary artery disease.

Table 2 Logistic Regression Analysis With Coronary Spastic Angina

Factor	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
Age	1.000	0.962–1.038	0.9864			
Hypertension	0.547	0.254–1.179	0.1235			
Diabetes mellitus	0.574	0.245–1.346	0.2013			
Cigarette smoking	2.528	1.152–5.549	0.0208	2.757	1.165–6.524	0.0210
TC	0.998	0.986–1.010	0.7420			
Log-TG	1.761	0.270–11.48	0.5541			
HDL-C	0.976	0.953–1.000	0.0470	0.985	0.960–1.011	0.2555
LDL-C	1.005	0.991–1.019	0.4960			
Fibrinogen	1.003	0.998–1.009	0.2415			
BMI	1.006	0.888–1.140	0.9213			
Creatinine	0.340	0.055–2.081	0.2429			
Log-CRP	0.935	0.343–2.554	0.8962			
Family history of CAD	1.537	0.615–3.838	0.3576			
Log-adiponectin	0.032	0.005–0.204	0.0003	0.036	0.005–0.253	0.0008

OR, odds ratio; CI, confidence interval. All other abbreviations, see Table 1.

Results

Table 1 shows the clinical and biochemical characteristics of both groups. Plasma log-adiponectin levels were significantly lower in the CSA group compared with the CPS group (0.61±0.28 vs 0.80±0.21 μg/ml, p<0.0001). The prevalence of cigarette smoking was significantly higher in the CSA group than in the CPS group. High-density lipoprotein cholesterol (HDL-C) levels were significantly lower in the CSA group than in the CPS group.

Multiple logistic regression analysis revealed that hypoadiponectinemia is a predictive risk factor for coronary artery spasm, even after adjustment for cigarette smoking and HDL-C, which were significant in the simple logistic regression (Table 2).

Discussion

The plasma adiponectin level is considered to be a marker of atheromatous vascular changes[7] and smoking is a significant predictive risk factor for coronary artery spasm[8] but the relationship between adiponectin and coronary artery spasm has not been determined to date. In this study, we showed that plasma adiponectin levels were significantly lower in the CSA group than in the CPS group and that low levels of plasma adiponectin were closely related to the presence of CSA, independent of cigarette smoking.

Endothelial dysfunction together with reduced endothelial vasodilatory function and smooth muscle hypercontraction in coronary arteries may play an important role in the pathogenesis of coronary artery spasm[1] In particular, NO, which is recognized as an endothelium-derived relaxing factor, is deficient in patients with CSA[1] Cigarette smoking is considered to be a major risk factor for CSA[1] because the oxidative stress associated with cigarette smoking may be a source of free radicals, which may cause coronary artery spasm by reducing NO activity[1] On the other hand, a recent report indicates that adiponectin stimulates the production of NO in vascular endothelial cells[2] and furthermore, that hypoadiponectinemia is associated with impaired endothelium-dependent vasorelaxation, based on significantly re-

duced ACh-induced vasorelaxation in adiponectin-knockout mice compared with wild-type mice.[4] In the present study, we demonstrated that adiponectin is a significant determinant of coronary artery spasm, irrespective of cigarette smoking, which suggests that the mechanism(s) by which hypoadiponectinemia impairs endothelium-dependent vasodilatation of the coronary arteries is different from that of cigarette smoking. Although the exact mechanism(s) of adiponectin-induced endothelium-dependent relaxation of coronary arteries is unknown, several potential mechanisms have been speculated. Hypoadiponectinemia is associated with insulin resistance and metabolic syndrome[9] and adiponectin may restore impaired endothelial vasomotor function through improvement of insulin resistance and its associated metabolic abnormalities. Adiponectin may also increase NO production in coronary vascular endothelial cells by activating adenosine monophosphate-activated protein kinase, which in turn promotes eNOS via activation of phosphatidylinositol 3-kinase-Akt-dependent pathways in endothelial cells.[10]

The potential limitation of this study is that the subjects were all men, who have significantly lower plasma adiponectin concentrations than BMI-adjusted women.[6,9] Further studies are warranted to evaluate the significance of plasma adiponectin concentrations in the prevalence of coronary artery spasm in women.

In conclusion, low adiponectin level is associated with CSA in men, independent of cigarette smoking, and hypoadiponectinemia is a potential important risk factor for CSA.

Acknowledgments

This study was supported in part by a Research Grant for Cardiovascular Disease (17C-2) from the Ministry of Health, Labor, and Welfare, Japan, a Grant-in-aid for Scientific Research (B-17390232) from the Ministry of Education, Science, and Culture, Japan, and the Smoking Research Foundation Grant for Biomedical Research, Japan. We gratefully acknowledge the technical assistance of Sachiyo Tanaka.

References

1. Yasue H, Kugiyama K. Coronary spasm: Clinical features and pathogenesis. *Intern Med* 1997; **36:** 760–765.
2. Matsuzawa Y. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. *Atheroscler Suppl* 2005; **6:** 7–14.
3. Agata J, Nagahara D, Kinoshita S, Takagawa Y, Momwa N, Yoshida D, et al. Angiotensin II receptor blocker prevents increased arterial stiffness in patients with essential hypertension. *Circ J* 2004; **68:** 1194–1198.
4. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 2003; **42:** 231–234.
5. Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, et al. Induction of coronary artery spasm by acetylcholine in patients with variant angina: Possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation* 1986; **74:** 955–963.
6. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257:** 79–83.
7. Kojima S, Funahashi T, Maruyoshi H, Honda O, Sugiyama S, Kawano H, et al. Levels of the adipocyte-derived plasma protein, adiponectin, have a close relationship with atheroma. *Thromb Res* 2005; **115:** 483–490.
8. Takaoka K, Yoshimura M, Ogawa H, Kugiyama K, Nakayama M, Shimasaki Y, et al. Comparison of the risk factors for coronary artery spasm with those for organic stenosis in a Japanese population: Role of cigarette smoking. *Int J Cardiol* 2000; **72:** 121–126.
9. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, et al. Adiponectin as a biomarker of the metabolic syndrome. *Circ J* 2004; **68:** 975–981.
10. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem* 2004; **279:** 1304–1309.

Hypertension
JOURNAL OF THE AMERICAN HEART ASSOCIATION
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Association of Hypoadiponectinemia With Smoking Habit in Men

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Hypertension 2005;45;1094-1100; originally published online May 16, 2005;
DOI: 10.1161/01.HYP.0000169444.05588.4c
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Abstract—Adiponectin is emerging as an important molecule in obesity, the metabolic syndrome, and cardiovascular disease. On the other hand, smoking habit is well known to be related to cardiovascular disease and hypertension. To examine the association between adiponectin concentration and smoking habit, we performed an epidemiological survey and an acute exposure test in humans and an experiment in adipocytes to elucidate the mechanism underlying the association between adiponectin and smoking. In the epidemiological study, we enrolled a total of 331 male subjects to examine chronic smoking exposure. Plasma adiponectin was significantly lower ($P=0.01$) in current smokers (5.3±0.3 μg/mL) than in never-smokers (6.5±0.4 μg/mL). A significant association between smoking and low adiponectin level was also confirmed in multiple regression analysis including age, body mass index, hypertension, diabetes, hyperlipidemia, and creatinine clearance (never-smokers 6.5±0.4 μg/mL; past smokers 5.6±0.3 μg/mL; current smokers 5.2±0.4 μg/mL; $F=4.52$; $P=0.01$). To examine the acute effect of smoking on adiponectin concentration for 12 hours, we measured plasma adiponectin level in 5 male never-smokers before smoking and 3, 6, and 12 hours after smoking, with the result that adiponectin showed a significant decrease after smoking (12 hours; −14.5±0.6%; $P<0.01$). In cultured mouse 3T3-L1 adipocytes, H_2O_2 and nicotine reduced the mRNA expression and secretion of adiponectin in a dose-dependent manner. Smoking habit is associated with adiponectin concentration in men, and its suppressive effect is mediated in part through direct inhibition of smoking on adiponectin expression in adipocytes. **(*Hypertension*. 2005;45:1094-1100.)**

Key Words: smoking ■ oxidative stress ■ risk factors ■ lipids ■ lipoprotein ■ metabolism

Cigarette smoking exacts a continuing toll on public health and is an established risk factor for hypertension and cardiovascular disease, and nonsmoking is a leading preventive strategy against coronary artery disease. Furthermore, cigarette smoking and its cessation are reported to alter lipid metabolism.[1–3] It is well established that smoking stimulates lipolysis in vivo. The lipolytic effect of smoking has been attributed to the nicotine component being mediated via release of catecholamines.[3] Nicotine, a major component of cigarette smoke, promotes inflammation[4] and progression of atherosclerotic lesions.[5,6] Furthermore, nicotine also has a direct effect on human adipose tissue.[7–9] On the other hand, oxidative stress has been shown to be a key phenomenon involved in the effects of smoking. Cigarette smoke contains a large amount of free radicals, which degrade NO released from the endothelium and also produce highly reactive intermediates, resulting in endothelial injury. Oxidative stress can damage many cell components, such as DNA, lipid membranes, and proteins, and lead to apoptosis and cell damage.[10,11]

Adiponectin, an adipose tissue–specific collagen-like factor, is abundantly present in plasma and possesses antiatherogenic properties. Adiponectin is emerging as an important molecule in obesity,[12] the metabolic syndrome,[13–15] cardiovascular disease,[16] lipid metabolism,[15] and hypertension.[17,18] In addition, adiponectin concentration is correlated independently with the vasodilator response to reactive hyperemia, and its concentration could be an independent parameter of endothelial function.[19] Endothelial dysfunction, an early marker of atherosclerosis, has been observed in chronic smokers as well as after acute cigarette smoking.[20,21] These results suggest that adiponectin may be a mediator between smoking and several diseases such as hypertension and coronary artery disease. Furthermore, smoking may directly regulate adiponectin concentration via lipolysis.

Although Miyazaki et al[22] reported that in subjects with coronary artery disease, smoking status was associated with reduced adiponectin concentration, using a small number of subjects, the association between plasma adiponectin and smoking status was evaluated without adjusting for con-

Received February 24, 2005; first decision March 19, 2005; revision accepted April 5, 2005.

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Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000169444.05588.4c